European Journal of Orthodontics 33 (2011) 365–371
doi:10.1093/ejo/cjq084
Advance Access Publication 5 October 2010

The masticatory system under varying functional load. Part 2: effect of reduced masticatory load on the degree and distribution of mineralization in the rabbit mandible

Thorsten Grünheid*,****, Geerling E. J. Langenbach**, Peter Brugman**, Vincent Everts*** and Andrej Zentner****

*Division of Orthodontics, University of Minnesota, Minneapolis, USA, Departments of **Functional Anatomy, ***Oral Cell Biology and ****Orthodontics, Academic Centre for Dentistry Amsterdam, Research Institute MOVE, University of Amsterdam and VU University Amsterdam, The Netherlands

Correspondence to: Dr Thorsten Grünheid, Division of Orthodontics, School of Dentistry, University of Minnesota, 6-326 Moos Tower, 515 Delaware Street SE, Minneapolis, MN 55455, USA. Email: tgruenhe@umn.edu

SUMMARY A reduction in mechanical loading of the mandible brought about by mastication of soft food is assumed to decrease the remodelling rate of bone, which, in turn, might increase the degree of bone mineralization.

The effect of a reduction in masticatory functional load on the degree and distribution of mineralization of mandibular bone was investigated in male juvenile New Zealand White rabbits. The experimental animals (n = 8) had been raised on a diet of soft pellets from 8 to 20 weeks of age, while the controls (n = 8) had been fed pellets of normal hardness. The degree of mineralization of bone (DMB) was assessed at the attachment sites of various jaw muscles, the condylar head, and the alveolar process. Differences between groups and among sites were tested for statistical significance using a Student's t-test and one-way analysis of variance, respectively.

The DMB did not differ significantly between the experimental and control animals at any of the sites assessed. However, in the rabbits that had been fed soft pellets, both cortical bone at the attachment sites of the temporalis and digastric muscles and cortical bone in the alveolar process had a significantly higher DMB than cortical bone at the attachment site of the masseter muscle, while there were no significant differences among these sites in the control animals.

The results suggest that a moderate reduction in masticatory functional load does not significantly affect the remodelling rate and the DMB in areas of the mandible that are loaded during mastication but might induce a more heterogeneous mineral distribution.

Introduction

Bone is a dynamic tissue, which continuously undergoes adaptive remodelling, i.e. resorption and apposition, to meet the requirements of its functional environment. The remodelling rate is a major determinant of the degree of mineralization of bone (DMB; Boivin and Meunier, 2002). A higher remodelling rate decreases the time available for secondary mineralization, which results in bone with a lower DMB (Boivin *et al.*, 2009).

The remodelling rate of bone is related to the magnitude of intermittent mechanical loading and the resulting dynamic strains in the tissue (Lisková and Hert, 1971; Turner, 1998). In general, more heavily loaded bone has a higher remodelling rate and is therefore less mineralized and less stiff than lower loaded bone (Rubin and Lanyon, 1985; Cullen *et al.*, 2001). Regional differences in the DMB of cortical bone have been described in a number of species (Riggs *et al.*, 1993; Loveridge *et al.*, 2004; van Ruijven *et al.*, 2007). This regionally heterogeneous organization of bone mineral has been attributed to regional differences in the magnitude and mode of strain brought about by mechanical loading (Skedros *et al.*, 1994).

Under physiological conditions, intermittent mechanical loading of bone is caused predominantly by muscle contractions. The muscles thus provide an important mechanical stimulus for bone remodelling by inducing strains in the skeletal system (Turner, 2000). The significance of muscle-generated bone loading is illustrated by the effect on the skeleton under conditions of increased or decreased muscle activity. For example, the loss of normal physiologic loading after spinal cord injury causes rapid severe bone loss in the paralyzed extremities of affected individuals, which can be counteracted by long-term electrical stimulation of muscles (Dudley-Javoroski and Shields, 2008).

In the masticatory system, long-term alterations in the pattern of muscular strains can be enforced by changing the consistency of the available food (Yamada and Kimmel, 1991; Kiliaridis *et al.*, 1996). For instance, the continuous intake of a soft diet during growth and development has been shown to reduce the functional capacity of jaw muscles (Kiliaridis and Shyu, 1988; Liu *et al.*, 1998) and to influence the morphology (Abed *et al.*, 2007; Ödman *et al.*, 2008; Enomoto *et al.*, 2010) and internal bone structure of the mandible (Bresin *et al.*, 1999). The reduction in intermittent mechanical loading of the mandible during mastication of a soft diet may also decrease the rate of bone remodelling (Bouvier and Hylander, 1981), which, in turn, would increase the DMB. As mechanical loading during mastication is not evenly distributed over the mandible, this increase might be regionally different. For instance, changes in the DMB as a result of altered mechanical stimulation might be most pronounced in areas where muscle contractions load mandibular bone directly, such as the attachment sites of the jaw muscles or in areas where muscle contractions create reaction forces, such as the alveolar process and temporomandibular joint.

The aim of this study was to investigate the effect of a reduction in masticatory load on the mineralization of mandibular bone. For this purpose, the degree and distribution of mineralization was assessed in mandibles of rabbits that had been fed diets of different physical consistency during late postnatal development. Since the DMB is assumed to be related to the mechanical loading generated by muscle contractions, it was hypothesized that the DMB of mandibular bone would show region-specific increases, especially at the sites of muscle attachment, in response to reduced food hardness.

Materials and methods

Animal experiment and tissue preparation

The animal experiment has been fully described in Part 1. In brief, 16 male New Zealand White rabbits were randomly divided into two equal-sized groups at the age of 8 weeks. The experimental group was fed a diet of soft pellets requiring significantly reduced peak loadings (10 N/cm^2) to break the pellet in comparison with the standard pellets (120 N/cm^2) fed to the control group. At 20 weeks of age, the animals were killed, their mandibles were dissected, carefully freed from soft tissues, and split in half at the symphysis. The tooth-bearing fragments were separated from the ascending rami by vertical cuts carried out dorsal to the crowns of the molars. Care was taken not to cut the bone at the attachment sites of the masseter and medial pterygoid muscles. All bone samples were obtained within 8 hours *post mortem* and stored in methanol at 4°C before analysis.

Degree and distribution of mineralization

The right hemimandibles were scanned in a micro-computed tomography system (CT 40; Scanco Medical AG, Brüttisellen, Switzerland) at an isotropic spatial resolution of 18 m, as described in detail elsewhere (Mulder *et al.*, 2004). The computed linear attenuation coefficient of the X-ray beam for each volume element (voxel) was represented by a grey value in the reconstruction. This attenuation coefficient is proportional to the local DMB (Nuzzo *et al.*, 2002; Mulder *et al.*, 2004).

The DMB was determined in eight predefined volumes of interest (VOI) of each hemimandible as the mass of the mineralized bone tissue relative to the volume of bone. This parameter is independent from the total volume or the amount of bone present in the VOI. The VOIs were selected at the attachment sites of the superficial masseter (M1–M3, ventral to dorsal), superficial temporalis, medial pterygoid, and digastric muscles, in the alveolar process adjacent to the second molar, and within the condylar head (Figure 1). The VOIs contained only cortical bone, except for that selected at the condylar head, which contained both cortical and cancellous bone.

Three-dimensional reconstructions of the VOIs were segmented to discriminate bone from background. The optimum thresholds for the VOIs were visually determined in four scans by gradual variation and comparison of the outcome with the original scan (Renders *et al.*, 2006). The mean values were applied as fixed thresholds to the segmentation of all VOIs to allow comparison of the samples (Ding *et al.*, 1999). This procedure was performed separately for the VOIs containing only cortical bone and those containing both cortical and cancellous bone. In a segmented image, only voxels with a linear attenuation value above the threshold, i.e. those representing bone, kept their original grey value, while voxels with a linear attenuation value below the threshold were transparent. The two outermost voxel layers characterized as bone were disregarded as these layers were likely to be corrupted by partial volume effects. Each grey value was then converted into a DMB value, using reference measurements of a calibration phantom containing hydroxyapatite in concentrations of 0, 50, 200, 800, and 1200 mg/cm^3 (QRM GmbH, Möhrendorf, Germany). The error of the method, determined as the relative difference between the measured and actual mineral density, was less than 3 per cent.

Statistical analysis

Mean values, standard deviations (SDs), coefficients of variation, and frequency distributions of the DMB were calculated for each VOI. The width of each distribution curve was calculated as twice the value of the SD. Differences between experimental and control groups were tested for statistical significance, for each VOI separately, using a Student's *t*-test, after the data had been tested for normality (Kolmogorov-Smirnov test). Differences among VOIs were tested for statistical significance, for each group of animals separately, using one-way analysis of variance with Holm-Sidak's method as the *post hoc* pairwise comparison

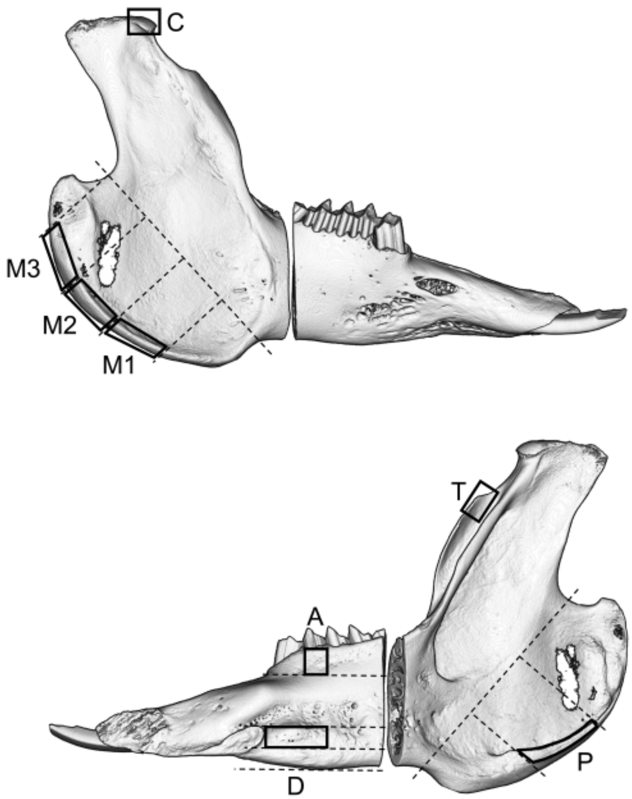

Figure 1 Lateral (top) and medial (bottom) views of a reconstructed right hemimandible showing the volumes of interest. C: condylar head. Selection cranial to the greatest medio-lateral extent and limited to the anterior fourth of the condylar process. M1–M3: attachment site of the superficial masseter muscle. Selections are bounded by lines perpendicularly intersecting a reference line, which connects the notches ventrocaudal and craniodorsal to the mandibular angle, at 25, 50, 75, and 100 per cent of its length. T: attachment site of the superficial temporalis muscle. A: alveolar bone. Selection medial to the second molar and cranial to a reference line parallel to the lower border of the mandible at the height of the incisor alveolar process. D: attachment site of the digastric muscle. Selection dorsal to the symphysis and between two reference lines parallel to the lower border of the mandible at 50 and 100 per cent of the distance between the lower border of the mandible and the most cranial part of the symphysis. P: attachment site of the medial pterygoid muscle. Selection is bounded by lines perpendicularly intersecting a reference line, which connects the notches ventrocaudal and craniodorsal to the mandibular angle, at 33 and 66 per cent of its length.

procedure. Statistical analyses were performed using SigmaStat 3.5 (Systat Software Inc., Point Richmond, California, USA) with P-values of less than 0.05 considered statistically significant.

Results

Mean values, SDs, and coefficients of variation of the DMB in the VOIs studied are shown in Table 1. Statistical testing revealed no significant differences between the experimental and control groups. However, in both the experimental and control groups, there were statistically significant differences in the DMB among the VOIs. In the experimental group, all VOIs containing only cortical bone, except for those in the mid- (M2) and dorsal (M3) parts of the attachment site of the masseter muscle, had a higher DMB than that selected within the condylar head. In addition, the attachment sites of the temporalis and digastric muscles and the alveolar bone medial to the second molar had a higher DMB than the dorsal part (M3) of the attachment site of the masseter muscle. The alveolar bone was also more highly mineralized than the mid-part (M2) of the attachment site of the masseter muscle. In the control group, all VOIs containing only cortical bone, except for that selected in the dorsal part (M3) of the attachment site of the masseter muscle, had a higher DMB than the VOI in the condylar head. These findings were similar to those in the experimental group. In contrast to the experimental group, there were no significant differences among the VOIs containing only cortical bone in the control group.

The frequency distribution curves of the DMB (Figure 2) did not differ significantly in width between the groups for any of the VOIs, suggesting that the degree of variation in DMB within the individual VOIs was similar in the experimental and control animals. The difference in their relative positions, i.e. the wider spread of the curves, indicated a greater heterogeneity in DMB among the sites studied in the experimental animals.

Table 1 Mean values ± standard deviations of degree of mineralization of bone (DMB) in the volumes of interest studied. HA, hydroxyapatite; CV, coefficient of variation.

	Experimental*		Control*	
	DMB (mg HA/cm^3)	CV (%)	DMB (mg HA/cm^3)	CV (%)
M1**	1151.32 ± 122.44^{a}	10.63	1171.37 ± 80.53^{a}	6.87
M2**	1093.10 ± 79.86^{b}	7.30	1116.08 ± 58.38^{b}	5.23
M3**	1034.55 ± 67.88^{c,d,e}	6.56	1076.34 ± 46.31	4.30
T**	1175.62 ± 77.01^{c,f}	6.55	1154.27 ± 94.94^{c}	8.22
P**	1150.04 ± 94.87^{g}	8.24	1183.04 ± 70.75^{d}	5.98
D**	1236.83 ± 108.63^{d,h}	8.78	1195.46 ± 146.38^{e}	12.24
A**	1277.96 ± 103.78^{b,e,i}	8.12	1212.80 ± 70.24^{f}	5.79
C**	987.60 ± 36.39^{a,f,g,h,i}	3.68	996.62 ± 34.63^{a,b,c,d,e,f}	3.47

*Statistically significant differences among volumes of interest, one-way analysis of variance $P < 0.05$.
**Volumes of interest selected at the attachment sites of the masseter (M1–M3), temporalis (T), medial pterygoid (P), and digastric (D) muscles, in the alveolar process (A) and within the condylar head (C). For detailed explanation, see Figure 1.
Within each column, groups depicted by the same superscript letters are statistically significantly different in *post hoc* pairwise comparison, Holm-Sidak's method $P < 0.05$.

Discussion

The present study investigated the effect of a masticatory functional change on the mineralization of mandibular bone. It was assumed that a reduction in intermittent

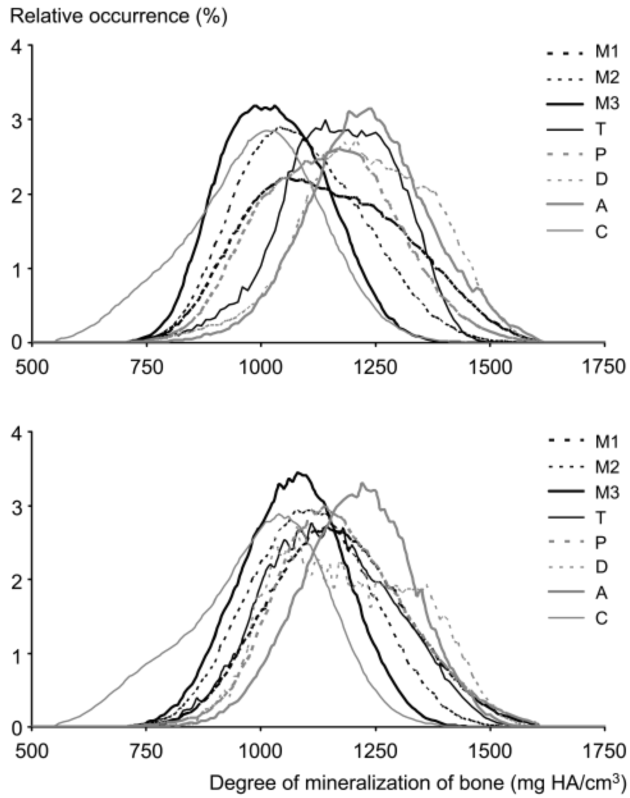

Figure 2 Mean distributions of the degree of mineralization of bone in the various volumes of interest studied in the experimental (top) and control (bottom) groups. For explanation of legend see Figure 1. HA, hydroxyapatite.

mechanical loading during mastication of a soft diet would decrease the rate of bone remodelling and increase the DMB. The results showed that reduced food hardness did not cause significant changes in the DMB at the examined sites. These findings differ from the results of other studies, which have shown that feeding diets of different consistency to growing rats might lead to a reduction in the rate of bone apposition (Yamada and Kimmel, 1991), resulting in lower bone mass (Bresin *et al.*, 1999) and alveolar bone density (Mavropoulos *et al.*, 2004, 2005) as well as in a higher degree of mineralization of mandibular bone (Tanaka *et al.*, 2007).

There are a number of possible reasons for this difference. One possibility is that the experimental period in the present research might have been too short to induce significant changes in the DMB. However, in the current study, the rabbits were fed diets of different consistency for 12 weeks, which, considering their life span, was comparable with experimental periods of 4–6 weeks typically used in similar studies on rats (Bresin *et al.*, 1999; Maki *et al.*, 2002; Mavropoulos *et al.*, 2004, 2005). Furthermore, changes in mandibular morphology resulting from decreased masticatory function have been reported in rabbits as early as 40 days after a reduction in dietary consistency (Tuominen *et al.*, 1993). Taking these considerations together, the duration of diet change in the present study was considered sufficiently long to induce changes in mandibular bone properties.

Another possible reason for the above difference in the findings might be a greater interindividual difference, which would mask the changes produced by the alteration in masticatory load, particularly with regard to the very small differences in the DMB between the experimental and control groups reported elsewhere (Tanaka *et al.*, 2007). This, however, is probably not the case because interindividual variation in the DMB (Table 1) was low, as shown by the low coefficients of variation, and was comparable with the results of the study of Tanaka *et al.* (2007). This finding supports the hypothesis proposed by Reid and Boyde (1987) that under physiological conditions, the rate of bone remodelling at a particular site can be considered a constant biological parameter.

Similar to earlier studies, the present investigation was carried out on juvenile animals as functional alterations influence bone tissue more effectively during adolescence (Parfitt, 1994). However, it has to be noted that changes in the properties of growing bones cannot, other than in a mature organism, solely be attributed to adaptive remodelling, i.e. resorption and apposition, but may be influenced by modelling, i.e. bone deposition during growth.

Most likely, the above disparity in results is based on the difference in food hardness used in various studies. Significant changes in mandibular bone properties in response to reduced food consistency have been reported in animals fed powdered (Maki *et al.*, 2002; Tanaka *et al.*, 2007) or liquefied (Yamada and Kimmel, 1991; Bresin *et al.*, 1999; Mavropoulos *et al.*, 2004, 2005) food. Although this experimental approach imposes greater differences in masticatory functional loads on experimental and control animals, it also alters more than just the dietary consistency. Powdered or liquefied food eliminates the need for mastication (Mavropoulos *et al.*, 2004) and changes the pattern of food uptake from incising and chewing into licking and sucking (Kitagawa *et al.*, 2004). In contrast to this experimental approach, the present study used purpose-made soft pellets, which did not change the feeding behaviour of the experimental animals. With regard to the difference in the consistency of the standard pellets fed to the control animals, these pellets mimic a 10-fold difference in the compressive strength between hard and soft foods normally eaten by humans (Yanagisawa *et al.*, 1985). The continuous intake of these pellets did not induce a significant alteration in the DMB in the present study. This result is in accordance with the finding of Maki *et al.* (2002) who, comparing powdered and kneaded diets with pellets of normal hardness, found a significantly different mandibular DMB in the animals fed a powdered diet, but not in those fed a kneaded diet with a consistency similar to the soft pellets used in the present study. Considering these findings, it appears that the DMB tends to increase only as a result of a significant reduction in masticatory activity induced by an unusually soft diet, such as a powdered diet.

The importance of bone remodelling in determining the average level of bone mineralization is generally accepted. In adult bone, the rate of remodelling is the major biological determinant of the DMB (Boivin *et al.*, 2009). The DMB increases when bone formation is suppressed and decreases when new bone formation is increased (Boivin and Meunier, 2002). The significantly higher DMB in the mandibles of rats raised on a powdered diet has been attributed to a reduction in the strain stimulus for new bone formation (Tanaka *et al.*, 2007). This reduction may have led to a preponderance of bone resorption as a result of disuse atrophy (Ferretti *et al.*, 2003), which is always observed in periods of physical inactivity (Forsén *et al.*, 1994). The findings of the present study suggest that the gentle loading during mastication of the softer pellets might have been sufficient exercise to prevent disuse atrophy. These considerations are in accordance with the finding that low-level mechanical signals can inhibit osteoclastic activity in the growing skeleton (Xie *et al.*, 2006).

Regional differences in the DMB have been reported for various bones and species (Riggs *et al.*, 1993; Loveridge *et al.*, 2004; van Ruijven *et al.*, 2007). The present investigation revealed significant differences in the DMB among the mandibular sites studied. In both the experimental and control groups, the DMB in the condylar head was lower than that at the cortical sites of the mandibular body, most probably because of the presence of cancellous bone in the condylar head. Differences in the DMB between cancellous and cortical mandibular bone are well documented (Mulder *et al.*, 2006; van Ruijven *et al.*, 2007; Willems *et al.*, 2007) and have been attributed to a higher remodelling rate in cancellous bone compared with cortical bone (Renders *et al.*, 2006). The DMB also differed significantly among cortical sites of the mandible but only in the experimental group. The attachment site of the masseter muscle was less highly mineralized than those of the temporalis and digastric muscles and the alveolar bone site. These findings suggest a greater heterogeneity in the DMB in the mandibles of the experimental animals.

Regional adaptations in material organization of bone reflect regional variations in strain magnitude (Skedros *et al.*, 1994). It is plausible that the attachment site of the masseter muscle, which is the main generator of force during mastication (Weijs *et al.*, 1989), is more heavily loaded than the attachment sites of the digastric and temporalis muscles or the bone of the alveolar process. In the present study, a lower DMB was found at the attachment site of the masseter muscle, which most likely resulted from a higher remodelling rate. It has been suggested that any adaptive remodelling influences the material properties of bone so as to achieve some mechanical advantage or to minimize material while maintaining a constant safety factor between peak functional stress and appropriate yield stress (Lanyon *et al.*, 1979). By rendering the bone more elastic, the lower DMB at the attachment site of the masseter muscle might constitute an advantage as it allows more bending of the bone during muscle contractions. The higher DMB at the attachment sites of the digastric and temporalis muscles and in the alveolar process might have been caused by suppression of bone formation relative to bone resorption. As it is advantageous to maintain bone weight as low as possible, this might reflect the body's endeavour to optimize energy use by minimizing the amount of material needed to maintain structural integrity under altered loading conditions.

Bone mineralization is influenced by the strain distribution in cortical and cancellous bone (van Ruijven *et al.*, 2007). The greater heterogeneity in the DMB in the experimental group might also have resulted from a relative strain distribution in the mandible, which was different from that in the control group. It is reasonable to assume that the intake of soft pellets led to less deformation of the mandible during mastication, and the local strains at the attachment sites of jaw muscles had, therefore, more influence on the DMB.

Adaptive responses depend on timing, duration, and intensity of a given stimulus. In the present investigation, the experimental stimulus, i.e. the reduction in masticatory load, induced significant changes in the phenotypic properties of the less recruited jaw muscles (see Part 1) but did not cause significant changes in the DMB of the less loaded mandibular bone. Studies using similar experimental stimuli have shown that reducing the mechanical loading of the mandible during growth and development can be effective in increasing the DMB in the mandible (Tanaka *et al.*, 2007). It seems, therefore, unlikely that the timing or the duration of the stimulus was ineligible to induce an adaptive change in the DMB of mandibular bone. However, it is possible that the stimulus was not sufficiently intense to cause significant changes in the mandibular DMB. The remodelling rate of mandibular bone, which is the main determinant of its DMB, might be under stronger genetic control and less easily influenced by environmental factors than the phenotypic properties of the jaw muscles. For this reason, a more intensive stimulus might be required to induce changes in the DMB of the mandible. Taken together, these considerations lend support to the idea that different intensities of a given stimulus might be necessary to modify the properties of muscular and skeletal craniofacial tissues.

Conclusions

The results of the present study suggest that a reduction in masticatory load within the range of physical consistency of foods eaten under normal life conditions does not strongly affect the DMB of mandibular bone in areas in which muscle contractions load mandibular bone directly or indirectly but might induce a more heterogeneous mineral distribution.

Funding

This work was supported by a European Orthodontic Society research grant.

Acknowledgements

The expert assistance of Leo van Ruijven is gratefully acknowledged.

References

Abed G, Buschang P H, Taylor R, Hinton R 2007 Maturational and functional related differences in rat craniofacial growth. Archives of Oral Biology 52: 1018–1025

Boivin G, Meunier P J 2002 Changes in bone remodeling rate influence the degree of mineralization of bone. Connective Tissue Research 43: 535–537

Boivin G, Farlay D, Bala Y, Doublier A, Meunier P J, Delmas P D 2009 Influence of remodeling on the mineralization of bone tissue. Osteoporosis International 20: 1023–1026

Bouvier M, Hylander W L 1981 Effect of bone strain on cortical bone structure in macaques (*Macaca mulatta*). Journal of Morphology 167: 1–12

Bredman J J, Wessels A, Weijs W A, Korfage J A M, Soffers C A, Moorman A F M 1991 Demonstration of 'cardiac-specific' myosin heavy chain in masticatory muscles of human and rabbit. The Histochemical Journal 23: 160–170

Bredman J J, Weijs W A, Korfage J A M, Brugman P, Moorman A F M 1992 Myosin heavy chain expression in rabbit masseter muscle during postnatal development. Journal of Anatomy 180: 263–274

Bresin A, Kiliaridis S, Strid K-G 1999 Effect of masticatory function on the internal bone structure in the mandible of the growing rat. European Journal of Oral Sciences 107: 35–44

Cullen D M, Smith R T, Akhter M P 2001 Bone-loading response varies with strain magnitude and cycle number. Journal of Applied Physiology 91: 1971–1976

Ding M, Odgaard A, Hvid I 1999 Accuracy of cancellous bone volume fraction measured by micro-CT scanning. Journal of Biomechanics 32: 323–326

Dudley-Javoroski S, Shields R K 2008 Asymmetric bone adaptations to soleus mechanical loading after spinal cord injury. Journal of Musculoskeletal and Neuronal Interactions 8: 227–238

English A W, Eason J, Schwartz G, Shirley A, Carrasco D I 1999 Sexual dimorphism in the rabbit masseter muscle: myosin heavy chain composition of neuromuscular compartments. Cells Tissues Organs 164: 179–191

Enomoto A, Watahiki J, Yamaguchi T, Irie T, Tachikawa T, Maki K 2010 Effects of mastication on mandibular growth evaluated by microcomputed tomography. European Journal of Orthodontics 32: 66–70

Ferretti J L, Cointry G R, Capozza R F, Frost H M 2003 Bone mass, bone strength, muscle-bone interactions, osteopenias and osteoporoses. Mechanisms of Ageing and Development 124: 269–279

Forsén L *et al.* 1994 Interaction between current smoking, leanness, and physical inactivity in the prediction of hip fracture. Journal of Bone and Mineral Research 9: 1671–1678

Galler S, Puchert E, Gohlsch B, Schmid D, Pette D 2002 Kinetic properties of cardiac myosin heavy chain isoforms in rat. Pflugers Archiv 445: 218–223

Grossman E J, Roy R R, Talmadge R J, Zhong H, Edgerton V R 1998 Effects of inactivity on myosin heavy chain composition and size of rat soleus fibres. Muscle and Nerve 21: 375–389

Grünheid T, Langenbach G E J, Korfage J A M, Zentner A, van Eijden T M G J 2009 The adaptive response of jaw muscles to varying functional demands. European Journal of Orthodontics 31: 596–612

Hancock M B 1986 Two-color immunoperoxidase staining: visualization of anatomic relationships between immunoreactive neural elements. American Journal of Anatomy 175: 343–352

He T, Olsson S, Daugaard J R, Kiliaridis S 2004 Functional influence of masticatory muscles on the fibre characteristics and capillary distribution in growing ferrets (*Mustela putonusfuro*)—a histochemical analysis. Archives of Oral Biology 49: 983–989

Henneman E 1981 Recruitment of motoneurons: the size principle. Progress in Clinical Neurophysiology 9: 26–60

Herring S W 2007 Masticatory muscles and the skull: a comparative perspective. Archives of Oral Biology 52: 296–299

Husom A D, Ferrington D A, Thompson L V 2005 Age-related differences in the adaptive potential of type I skeletal muscle fibers. Experimental Gerontology 40: 227–235

Ingervall B, Ridell A, Thilander B 1979 Changes in activity of the temporal, masseter and lip muscles after surgical correction of mandibular prognathism. International Journal of Oral Surgery 8: 290–300

Jarvis J C, Mokrusch T, Kwende M M N, Sutherland H, Salmons S 1996 Fast to slow transformation in stimulated rat muscle. Muscle and Nerve 19: 1469–1475

Kemsley E K, Defernez M, Sprunt J C, Smith A C 2003 Electromyographic responses to prescribed mastication. Journal of Electromyography and Kinesiology 13: 197–207

Kiliaridis S, Shyu B C 1988 Isometric muscle tension generated by masseter stimulation after prolonged alteration of the consistency of the diet fed to growing rats. Archives of Oral Biology 33: 467–472

Kiliaridis S, Engström C, Thilander B 1988 Histochemical analysis of masticatory muscle in the growing rat after prolonged alteration in the consistency of the diet. Archives of Oral Biology 33: 187–193

Kiliaridis S, Bresin A, Holm J, Strid K-G 1996 Effects of masticatory muscle function on bone mass in the mandible of the growing rat. Acta Anatomica 155: 200–205

Kitagawa Y, Mitera K, Ogasawara T, Nojyo Y, Miyauchi K, Sano K 2004 Alterations in enzyme histochemical characteristics of the masseter muscle caused by long-term soft diet in growing rabbits. Oral Diseases 10: 271–276

Korfage J A M, Koolstra J H, Langenbach G E J, van Eijden T M G J 2005 Fiber-type composition of the human jaw muscles—(part 2) role of hybrid fibres and factors responsible for inter-individual variation. Journal of Dental Research 84: 784–793

Korfage J A M, van Wessel T, Langenbach G E J, Ay F, van Eijden T M G J 2006a Postnatal transitions in myosin heavy chain isoforms of the rabbit superficial masseter and digastric muscle. Journal of Anatomy 208: 743–751

Korfage J A M, van Wessel T, Langenbach G E J, van Eijden T M G J 2006b Heterogeneous postnatal transitions in myosin heavy chain isoforms within the rabbit temporalis muscle. The Anatomical Record. Part A, Discoveries in Molecular, Cellular, and Evolutionary Biology 288: 1095–1104

Korfage J A M, Helmers R, de Goüyon Matignon M, van Wessel T, Langenbach G E J, van Eijden T M G J 2009 Postnatal development of fiber type composition in rabbit jaw and leg muscles. Cells Tissues Organs 190: 42–52

Langenbach G E J, van de Pavert S, Savalle W P M, Korfage J A M, van Eijden T M G J 2003 Influence of food consistency on the rabbit masseter muscle fibres. European Journal of Oral Sciences 111: 81–84

Langenbach G E J, van Wessel T, Brugman P, van Eijden T M G J 2004 Variation in daily masticatory muscle activity in the rabbit. Journal of Dental Research 83: 55–59

Lanyon L E, Magee P T, Baggott D G 1979 The relationship of functional stress and strain to the processes of bone remodelling. An experimental study on the sheep radius. Journal of Biomechanics 12: 593–600

Larsson L, Moss R L 1993 Maximum velocity of shortening in relation to myosin isoform composition in single fibers from human skeletal muscles. Journal of Physiology 472: 595–614

Lisková M, Hert J 1971 Reaction of bone to mechanical stimuli. Part 2. Periosteal and endosteal reaction of tibial diaphysis in rabbit to intermittent loading. Folia Morphologica 19: 301–317

Liu Z J, Ikeda K, Harada S, Kasahara Y, Ito G 1998 Functional properties of jaw and tongue muscles in rats fed a liquid diet after being weaned. Journal of Dental Research 77: 366–376

Loveridge N, Power J, Reeve J, Boyde A 2004 Bone mineralization density and femoral neck fragility. Bone 35: 929–941

Maeda N *et al.* 1987 Effects of long-term intake of a fine-grained diet on the mouse masseter muscle. Acta Anatomica 128: 326–333

Maki K, Nishioka T, Shioiri E, Takahashi T, Kimura M 2002 Effects of dietary consistency on the mandible of rats at the growth stage: computed X-ray densitometric and cephalometric analysis. The Angle Orthodontist 72: 468–475

Mavropoulos A, Kiliaridis S, Bresin A, Ammann P 2004 Effect of different masticatory functional and mechanical demands on the structural adaptation of the mandibular alveolar bone in young growing rats. Bone 35: 191–197

Mavropoulos A, Ammann P, Bresin A, Kiliaridis S 2005 Masticatory demands induce region-specific changes in mandibular bone density in growing rats. The Angle Orthodontist 75: 625–630

Maxwell L C, McNamara J A Jr, Carlson D S, Faulkner J A 1980 Histochemistry of fibres of masseter and temporalis muscles of edentulous monkeys *Macaca mulatta*. Archives of Oral Biology 25: 87–93

McCall G E, Byrnes W C, Dickinson A, Pattany P M, Fleck S J 1996 Muscle fiber hypertrophy, hyperplasia, and capillary density in college men after resistance training. Journal of Applied Physiology 81: 2004–2012

Mulder L, Koolstra J H, van Eijden T M G J 2004 Accuracy of microCT in the quantitative determination of the degree and distribution of mineralization in developing bone. Acta Radiologica 45: 769–777

Mulder L, van Groningen L B, Potgieser Y A, Koolstra J H, van Eijden T M G J 2006 Regional differences in architecture and mineralization of developing mandibular bone. The Anatomical Record. Part A. Discoveries in Molecular, Cellular, and Evolutionary Biology 288: 954–961

Negoro T *et al.* 2001 Histochemical study of rabbit masseter muscle: the effect of the alteration of food on the muscle fibers. Oral Medicine and Pathology 6: 65–71

Nuzzo S, Peyrin F, Cloetens P, Baruchel J, Boivin G 2002 Quantification of the degree of mineralization of bone in three dimensions using synchrotron radiation microtomography. Medical Physics 29: 2676–2681

Ödman A, Mavropoulos A, Kiliaridis S 2008 Do masticatory functional changes influence the mandibular morphology in adult rats. Archives of Oral Biology 53: 1149–1154

Oishi Y, Ishihara A, Yamamoto H, Miyamoto E 1998 Hindlimb suspension induces the expression of multiple myosin heavy chain isoforms in single fibres of the rat soleus muscle. Acta Physiologica Scandinavica 162: 127–134

Parfitt A M 1994 The two faces of growth: benefits and risks to bone integrity. Osteoporosis International 4: 382–398

Pellegrino M A, Canepari M, Rossi R, D'Antona G, Reggiani C, Bottinelli R 2003 Orthologous myosin isoforms and scaling of shortening velocity with body size in mouse, rat, rabbit and human muscles. Journal of Physiology 546: 677–689

Pette D 2002 The adaptive potential of skeletal muscle fibers. Canadian Journal of Applied Physiology 27: 423–448

Pette D, Staron R S 2001 Transitions of muscle fibre phenotypic profiles. Histochemistry and Cell Biology 115: 359–372

Raustia A M, Salonen M A M, Pyhtinen J 1996 Evaluation of masticatory muscles of edentulous patients by computed tomography and electromyography. Journal of Oral Rehabilitation 23: 11–16

Reader M, Schwartz G, English A W 2001 Brief exposure to testosterone is sufficient to induce sex differences in the rabbit masseter muscle. Cells Tissues Organs 169: 210–217

Reid S A, Boyde A 1987 Changes in the mineral density distribution in human bone with age: image analysis using backscattered electrons in the SEM. Journal of Bone and Mineral Research 2: 13–22

Renders G A P, Mulder L, van Ruijven L J, van Eijden T M G J 2006 Degree and distribution of mineralization in the human mandibular condyle. Calcified Tissue International 79: 190–196

Riggs C M, Vaughan L C, Evans G P, Lanyon L E, Boyde A 1993 Mechanical implications of collagen fibre orientation in cortical bone of the equine radius. Anatomy and Embryology 187: 239–248

Roy R R, Baldwin K M, Edgerton V R 1991 The plasticity of skeletal muscle: effects of neuromuscular activity. Exercise and Sport Sciences Reviews 19: 269–312

Rubin C T, Lanyon L E 1985 Regulation of bone mass by mechanical strain magnitude. Calcified Tissue International 37: 411–417

Sant'ana Pereira J A, Wessels A, Nijtmans L, Moorman A F M, Sargeant A J 1995 New method for the accurate characterization of single human skeletal muscle fibres demonstrates a relation between mATPase and MyHC expression in pure and hybrid fibre types. Journal of Muscle Research and Cell Motility 16: 21–34

Schiaffino S, Reggiani C 1994 Myosin isoforms in mammalian skeletal muscle. Journal of Applied Physiology 77: 493–501

Sciote J J, Kentish J C 1996 Unloaded shortening velocities of rabbit masseter muscle fibres expressing skeletal or alpha-cardiac myosin heavy chains. Journal of Physiology 492: 659–667

Scutter S D, Türker K S 1998 Recruitment stability in masseter motor units during isometric voluntary contractions. Muscle and Nerve 21: 1290–1298

Sfondrini G, Reggiani C, Gandini P, Bovenzi R, Pellegrino M A 1996 Adaptations of masticatory muscles to a hyperpropulsive appliance in the rat. American Journal of Orthodontics and Dentofacial Orthopedics 110: 612–617

Skedros J G, Bloebaum R D, Mason M W, Bramble D M 1994 Analysis of a tension/compression skeletal system: possible strain-specific differences in the hierarchical organization of bone. The Anatomical Record 239: 396–404

Svensson P, Wang K, Sessle B J, Arendt-Nielsen L 2004 Associations between pain and neuromuscular activity in the human jaw and neck muscles. Pain 109: 225–232

Tanaka E *et al.* 2007 Effect of food consistency on the degree of mineralization in the rat mandible. Annals of Biomedical Engineering 35: 1617–1621

Tuominen M, Kantomaa T, Pirttiniemi P 1993 Effect of food consistency on the shape of the articular eminence and the mandible. Acta Odontologica Scandinavica 51: 65–72

Turner C H 1998 Three rules for bone adaptation to mechanical stimuli. Bone 23: 399–407

Turner C H 2000 Muscle-bone interactions, revisited. Bone 27: 339–340

Urushiyama T, Akutsu S, Miyazaki J-I, Fukui T, Diekwisch T G H, Yamane A 2004 Change from a hard to soft diet alters the expression of insulin-like growth factors, their receptors, and binding proteins in association with atrophy in adult mouse masseter muscle. Cell and Tissue Research 315: 97–105

van Ruijven L J, Mulder L, van Eijden T M G J 2007 Variations in mineralization affect the stress and strain distributions in cortical and trabecular bone. Journal of Biomechanics 40: 1211–1218

van Wessel T *et al.* 2005 Fibre-type composition of rabbit jaw muscles is related to their daily activity. European Journal of Neurosciences 22: 2783–2791

Weijs W A, Brugman P, Grimbergen C A 1989 Jaw movements and muscle activity during mastication in growing rabbits. The Anatomical Record 224: 407–416

Widmer C G, Carrasco D I, English A W 2003 Differential activation of neuromuscular compartments in the rabbit masseter muscle during different oral behaviors. Experimental Brain Research 150: 297–307

Willems N M B K, Mulder L, Langenbach G E J, Grünheid T, Zentner A, van Eijden T M G J 2007 Age-related changes in microarchitecture and mineralization of cancellous bone in the porcine mandibular condyle. Journal of Structural Biology 158: 421–427

Xie L *et al.* 2006 Low-level mechanical vibrations can influence bone resorption and bone formation in the growing skeleton. Bone 39: 1059–1066

Yamada K, Kimmel D B 1991 The effect of dietary consistency on bone mass and turnover in the growing rat mandible. Archives of Oral Biology 36: 129–138

Yanagisawa Y, Tamura A, Akasaka M, Teramoto Y 1985 Physical properties of food and eating functions. 1: an objective method for the measurement of the physical properties of foods, and classification of foods. The Japanese Journal of Pedodontics 23: 962–983